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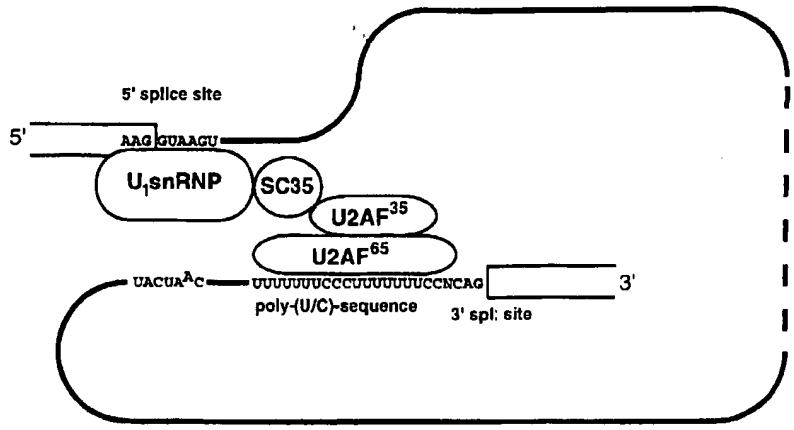
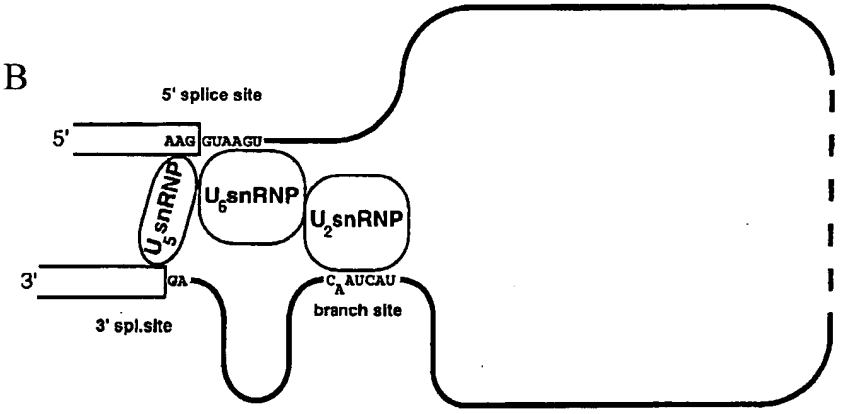
PATENT & TRADEMARK OFFICE

FIG. 1A

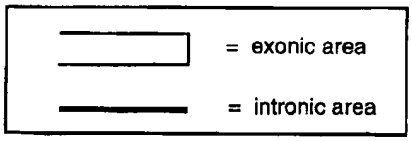


association between the 5' splice site and the 3' splice site in the E-complex

FIG. 1B



association between the 5' splice site and the 3' splice site in the B/C-complex



association between the 5' and 3' splice site at different points during splicing

FIG. 2A

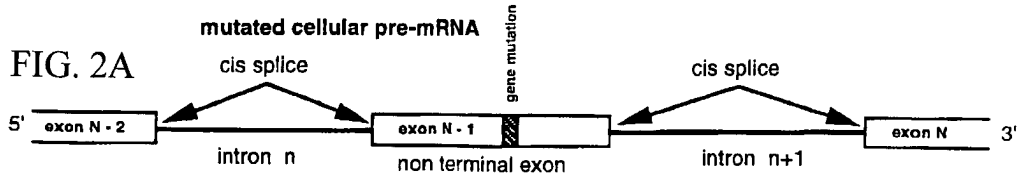


FIG. 2B

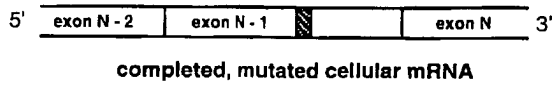


FIG. 3A

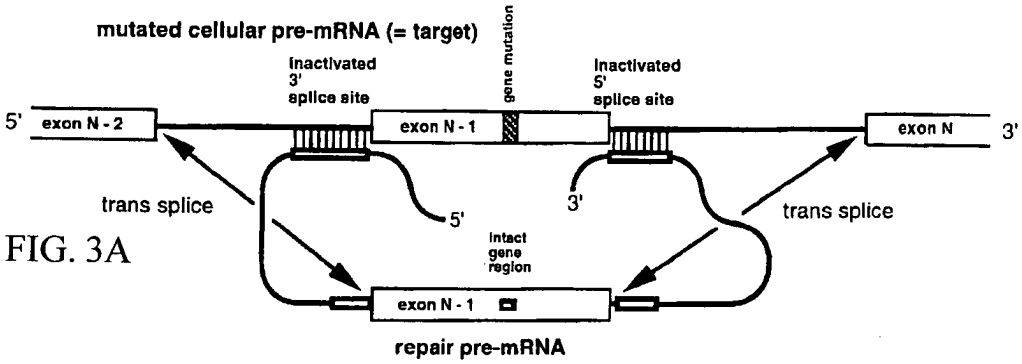


FIG. 3B

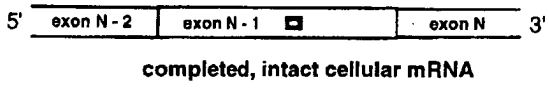


FIG. 4

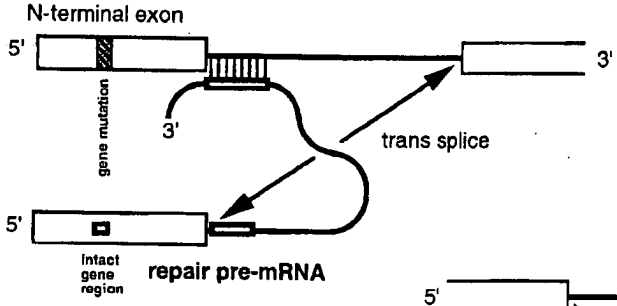
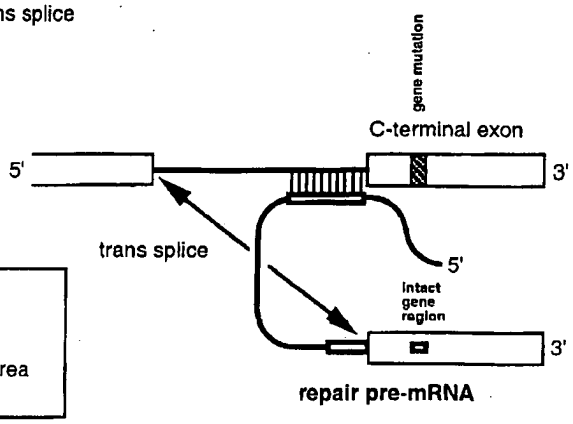
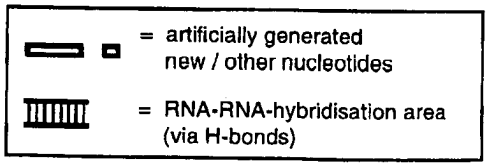


FIG. 5





repair of internal, N- or C-terminal gene mutated exons by trans splicing

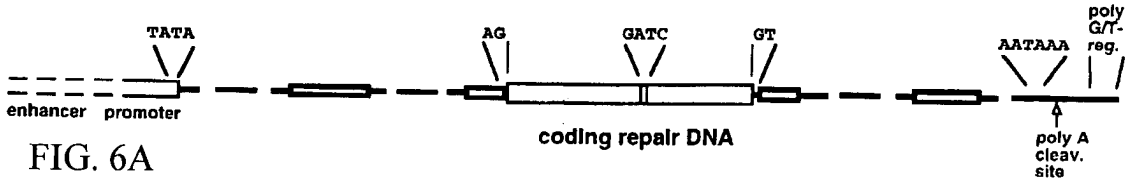


FIG. 6A

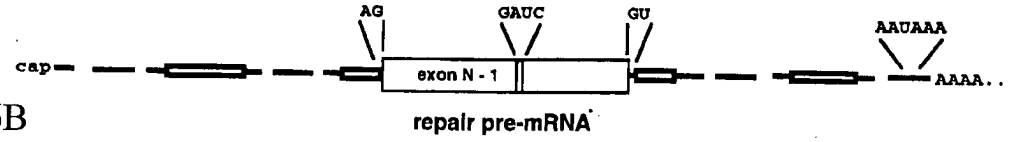


FIG. 6B

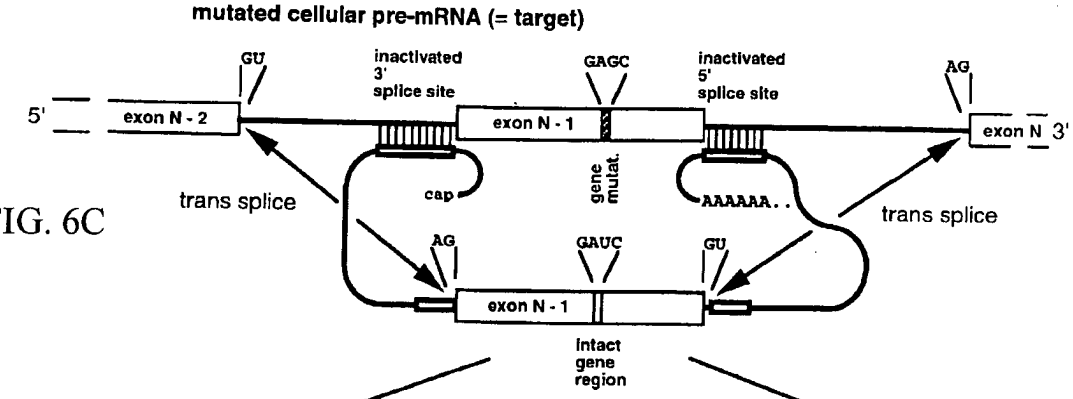


FIG. 6C

2 x trans splice reaction

cis splice reaction

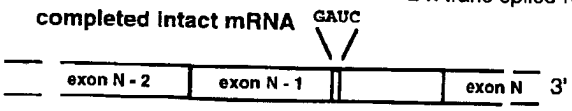


FIG. 6D

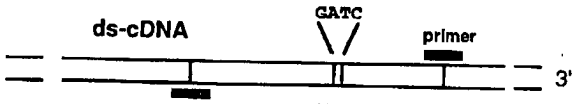


FIG. 6E

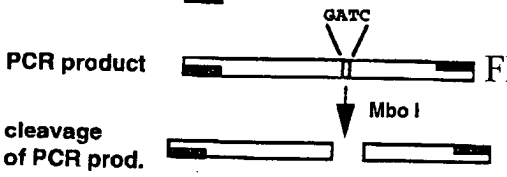


FIG. 6F

FIG. 6G

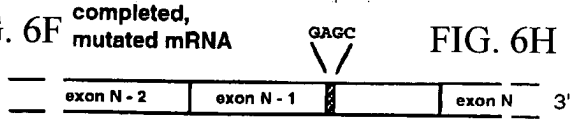


FIG. 6H

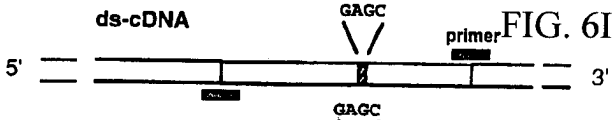


FIG. 6I

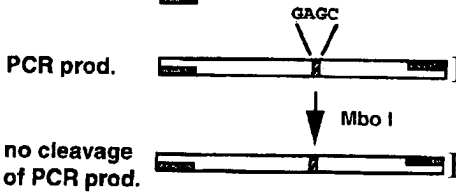


FIG. 6J

FIG. 6K

identification of repaired (intact) and not repaired (mutated) mRNA

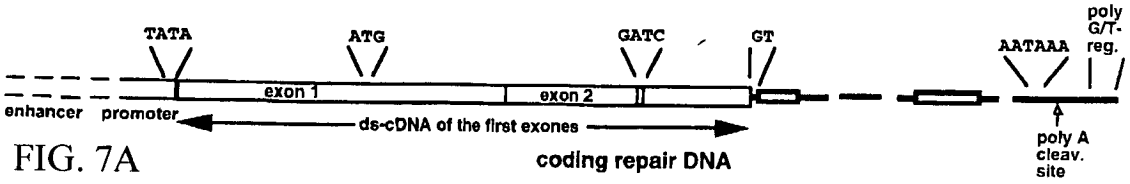


FIG. 7A

coding repair DNA

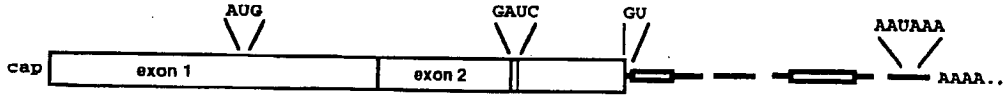


FIG. 7B repair pre-mRNA (with the first exons) and with a potent 5' splice site

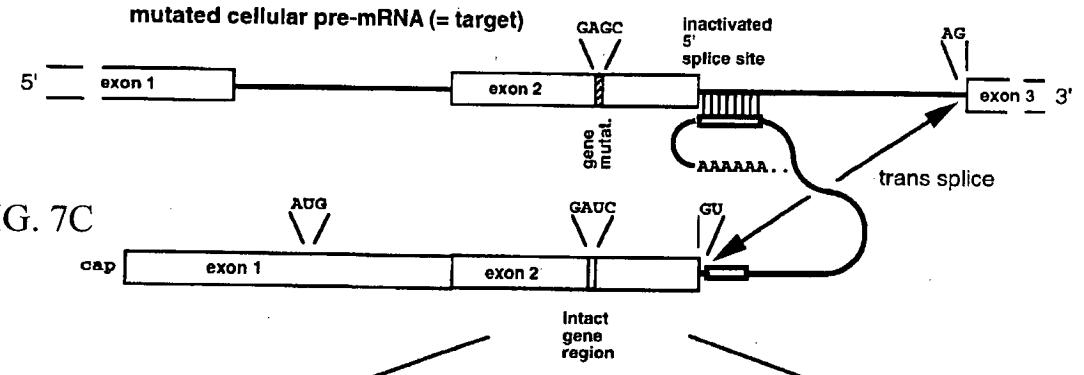


FIG. 7C

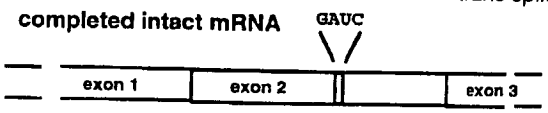


FIG. 7D

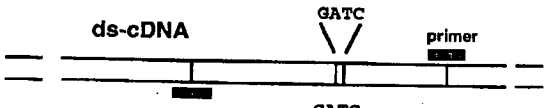


FIG. 7E

FIG. 7F

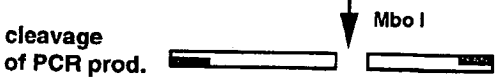


FIG. 7G

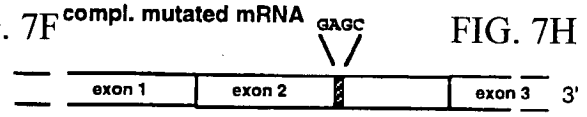


FIG. 7H

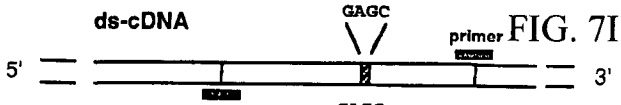


FIG. 7I

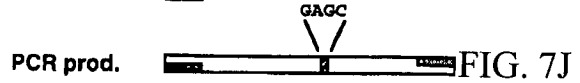


FIG. 7J

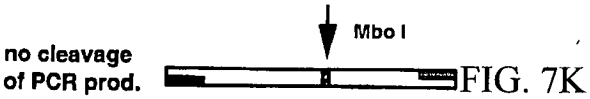


FIG. 7K

identification of repaired (intact) and not repaired (mutated) mRNA

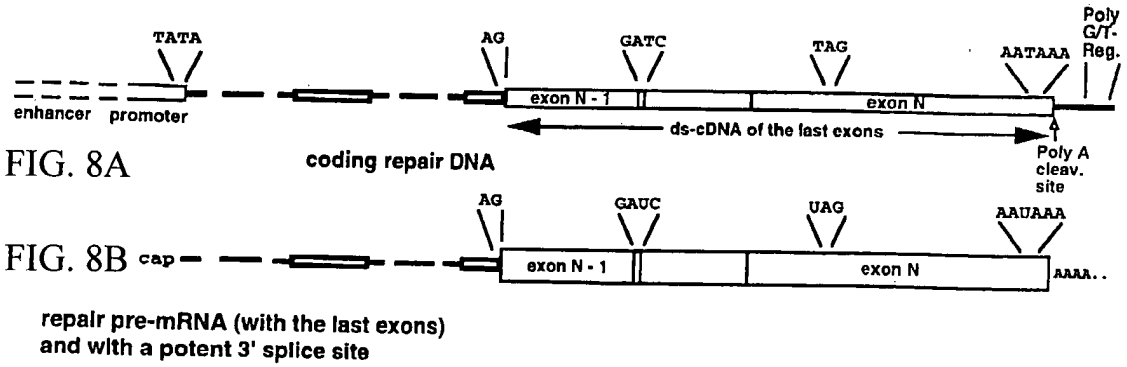


FIG. 8A

FIG. 8B

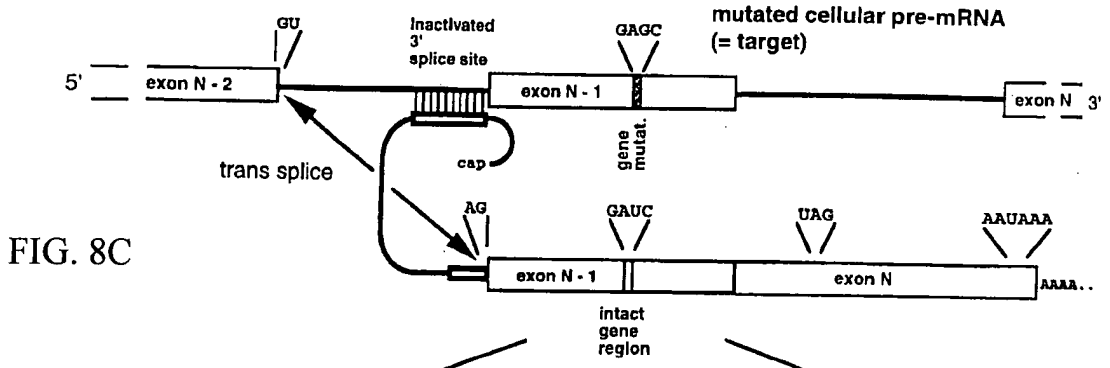


FIG. 8C

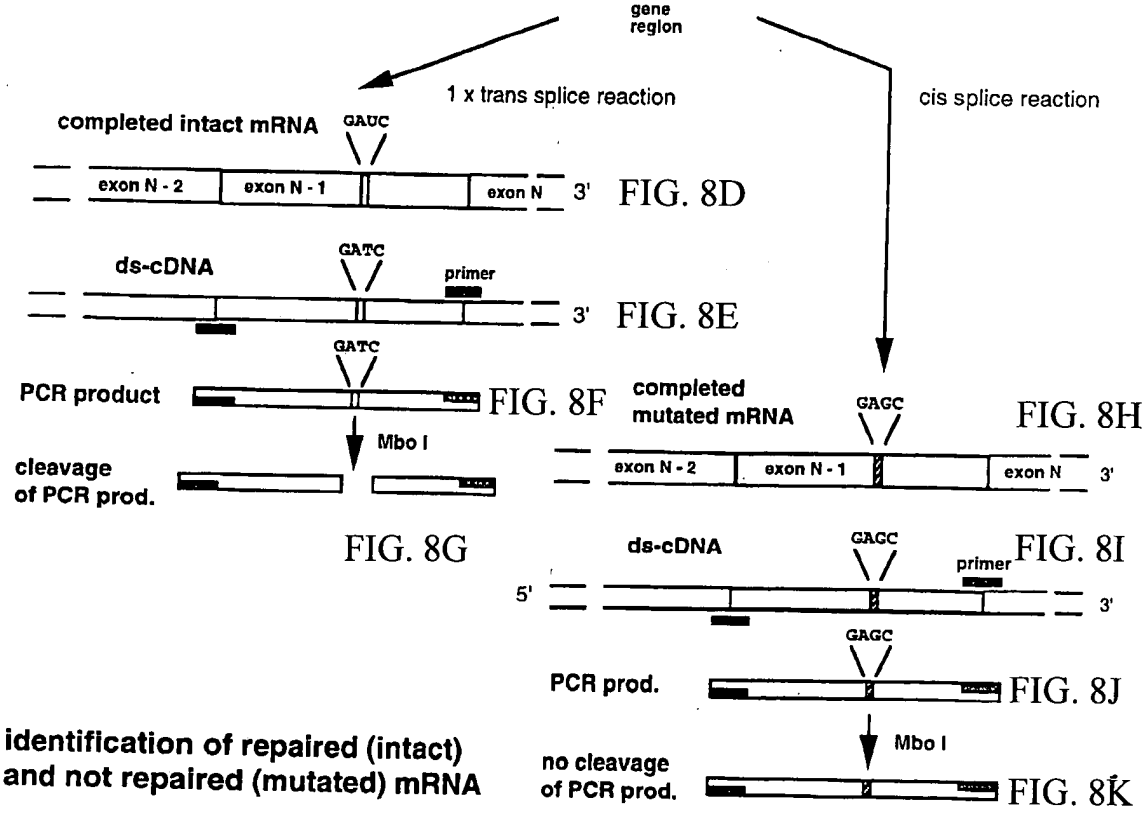


FIG. 8D

FIG. 8E

FIG. 8F

FIG. 8G

FIG. 8H

FIG. 8I

FIG. 8J

FIG. 8K

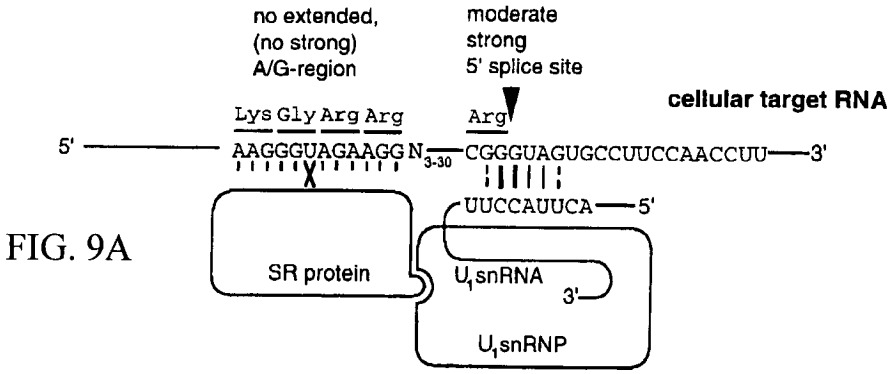


FIG. 9A

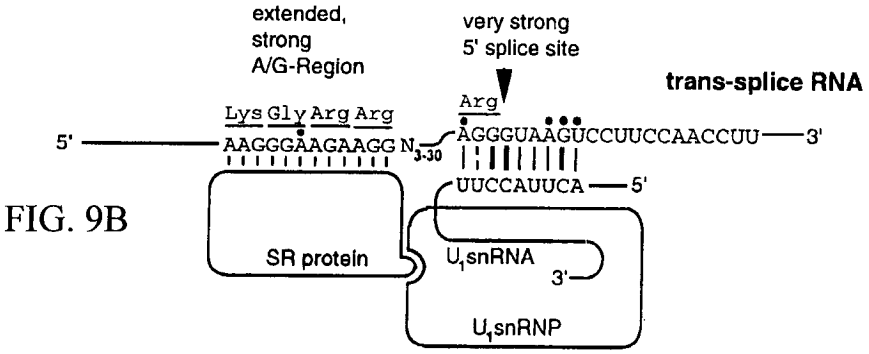


FIG. 9B

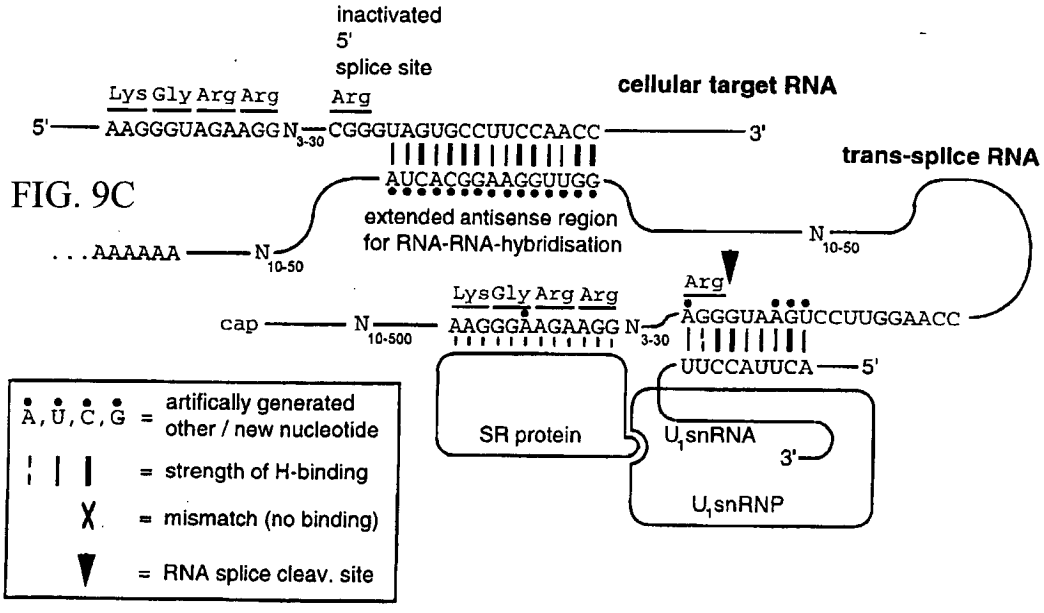


FIG. 9C

methodes to prefer the use of a 5' splice site on a trans-splice RNA

FIG. 10A

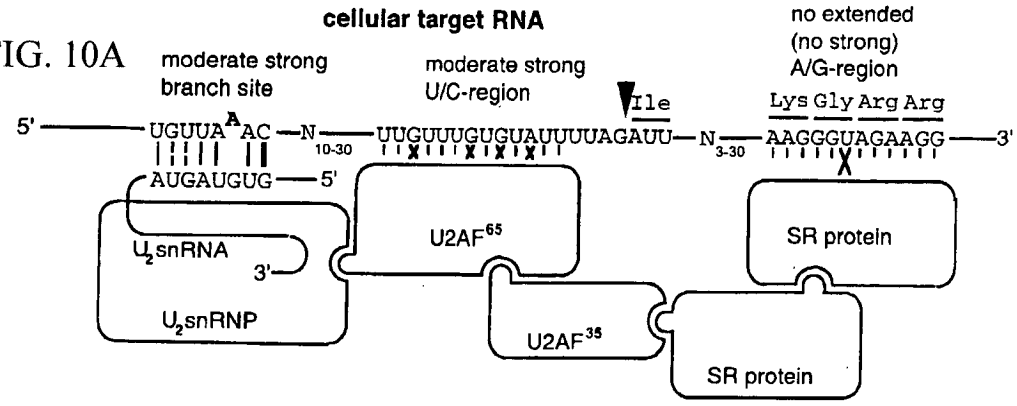


FIG. 10B

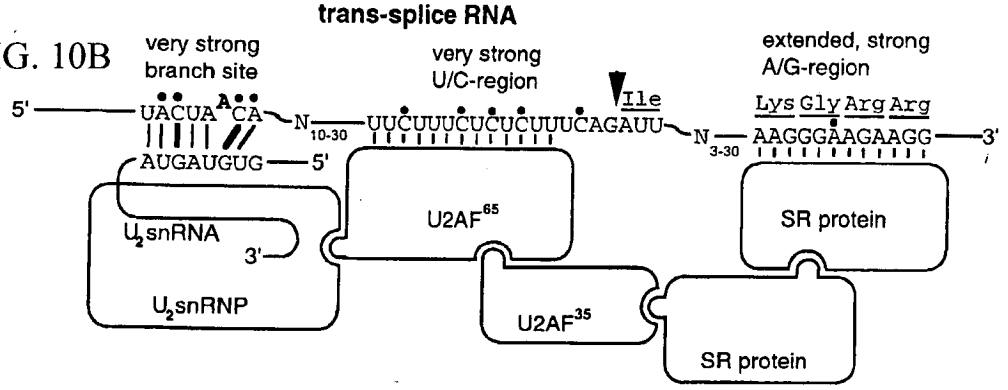
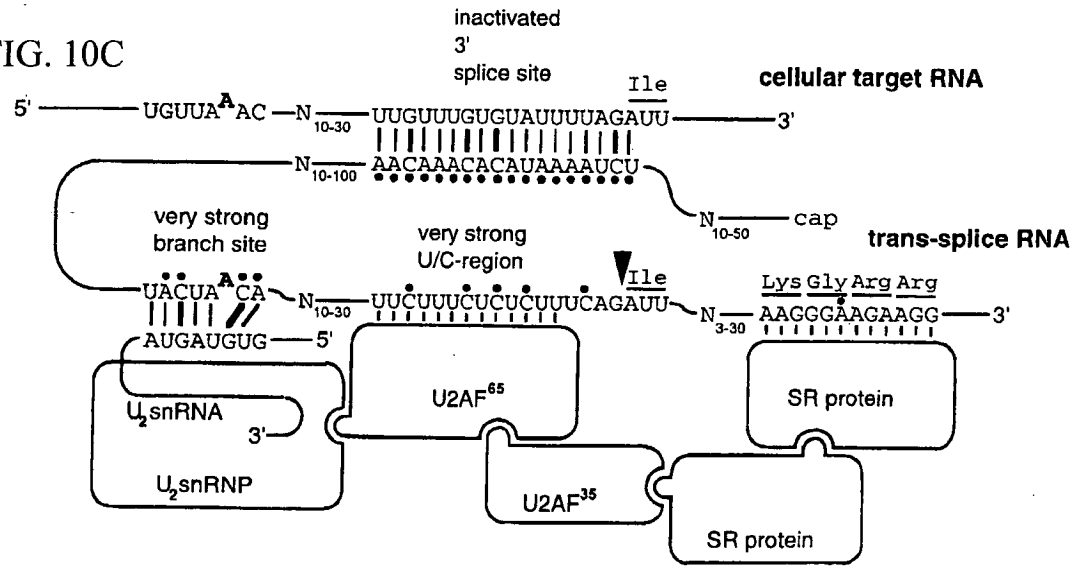


FIG. 10C



methodes to prefer the use of a 3' splice site on a trans-splice RNA

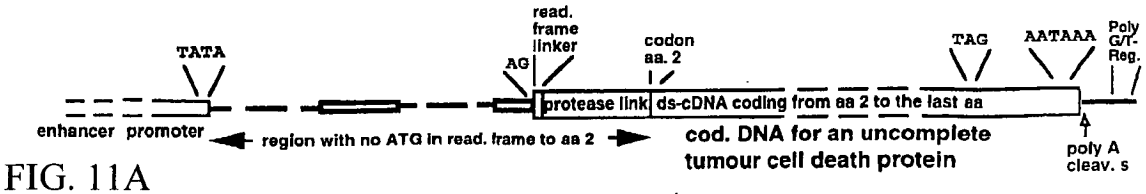


FIG. 11A

FIG. 11B

(yet) uncomplete tumour cell death pre-mRNA (with 2 linkers and codons for aminoacid (aa) 2 an the follow. aa and with a potent 3' splice site)

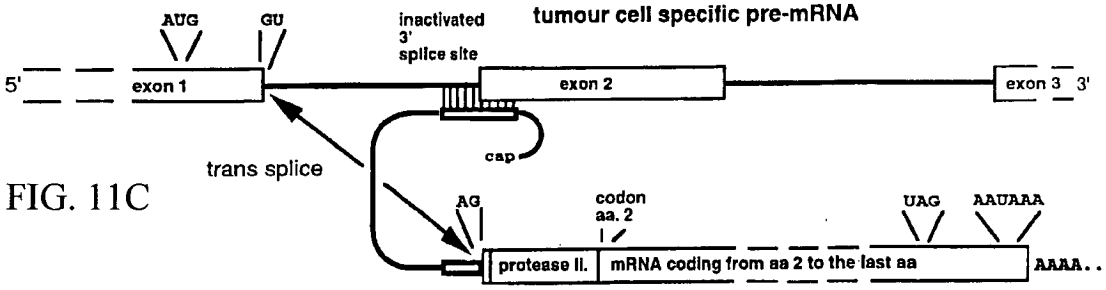


FIG. 11C

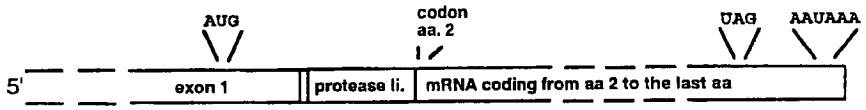


FIG. 11D

completed (active) hybrid mRNA with AUG start codon

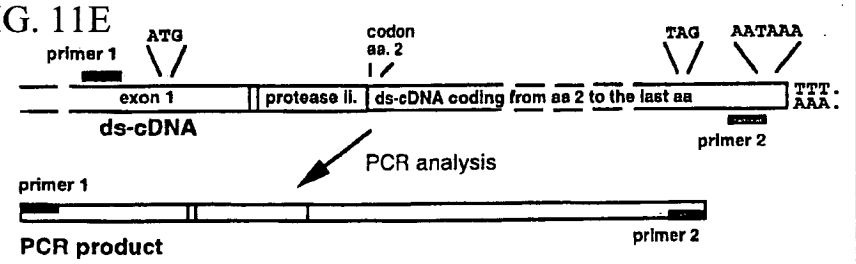


FIG. 11E

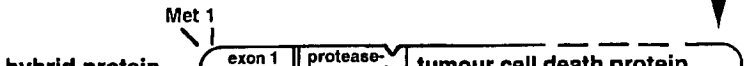


FIG. 11F

FIG. 11G

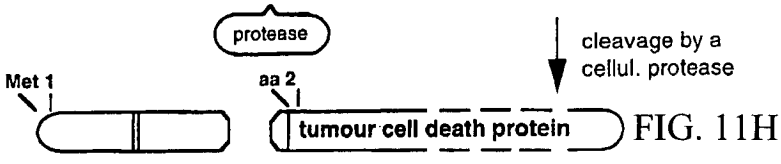


FIG. 11H

generation of deadly proteins in tumour cells after trans splicing

FIG. 12A

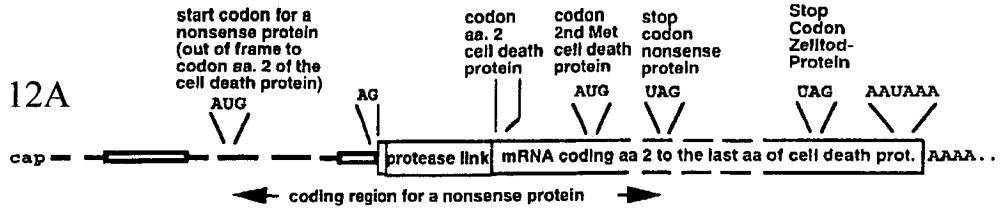


FIG. 12B

FIG. 13A

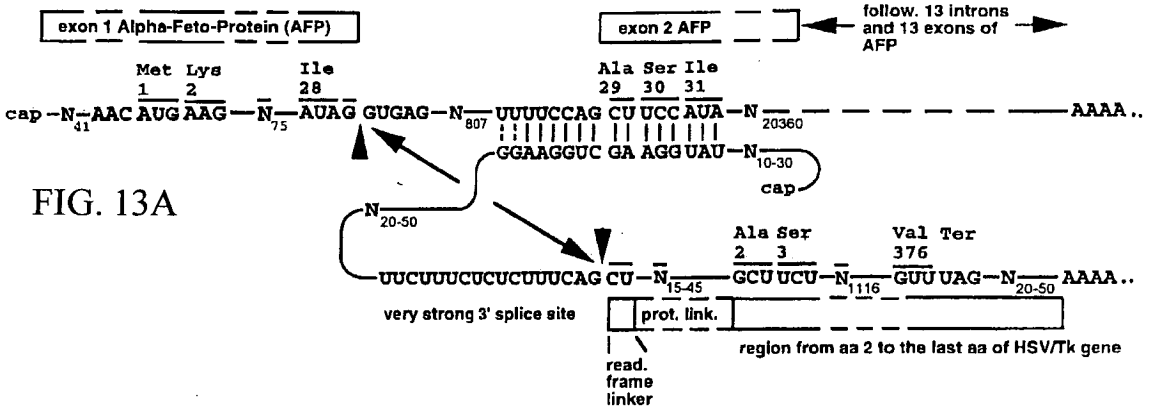


FIG. 13B

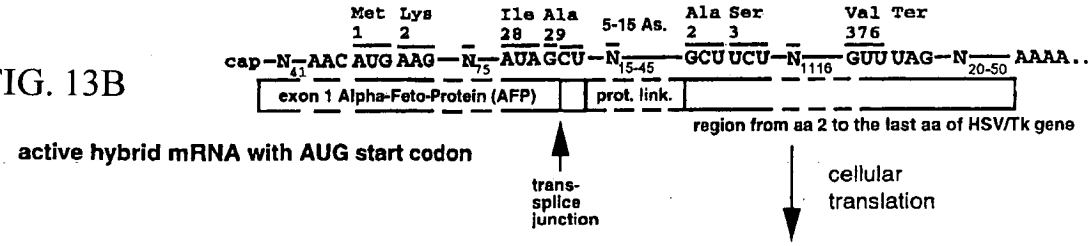


FIG. 13C

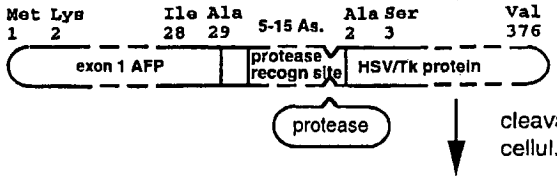
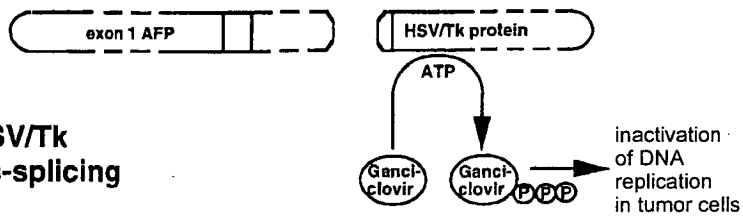


FIG. 13D



generation of active HSV/Tk protein after RNA trans-splicing

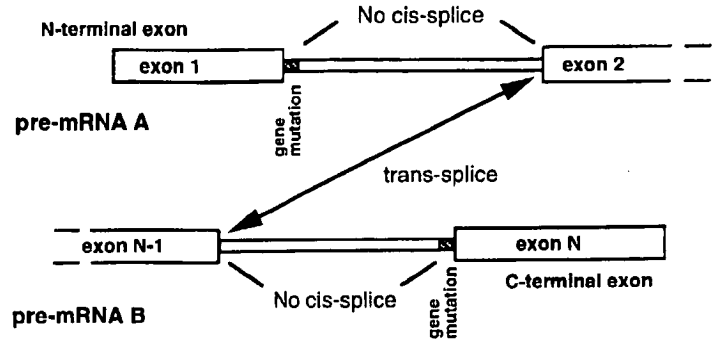


FIG. 14A

stimulation of trans-splicing
by mutations in the cis splice sites in N- or C-terminal introns

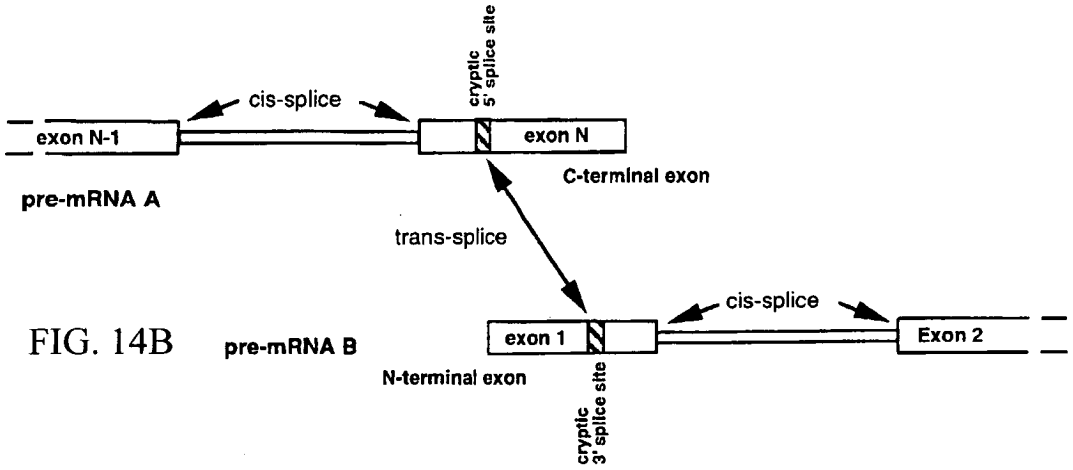


FIG. 14B

stimulation of trans-splicing
by activating cryptic splice sites in N- or C-terminal exons

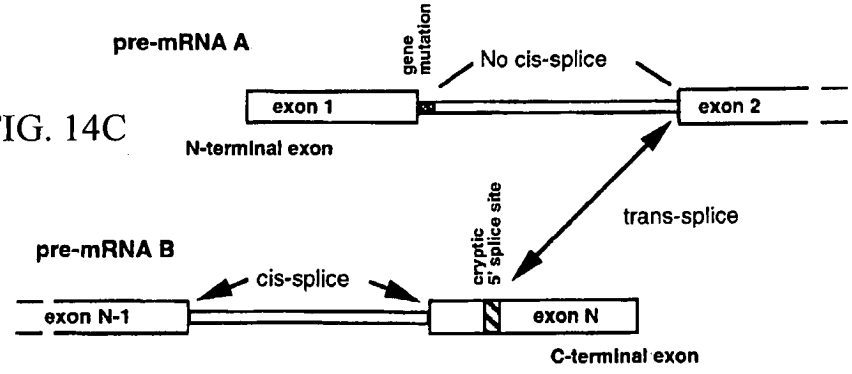
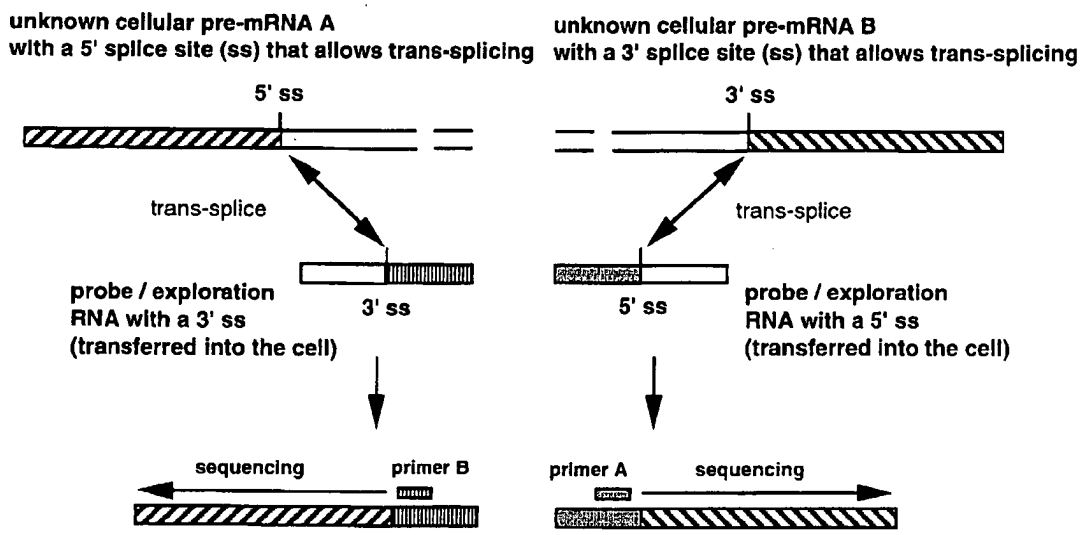


FIG. 14C

stimulation of trans-splicing by activating a cryptic splice site in N- or C-terminal exon and a mutation in a cis-splice site in a N-terminal intron

FIG. 15A

step 1: idendification potential pre-mRNAs for RNA trans-splicing

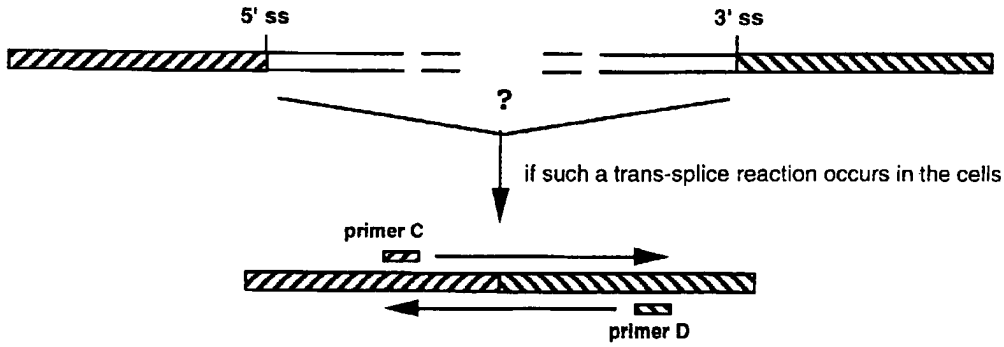


trans-splice product of probe / exploration RNA and cellular pre-mRNA A
(PCR sequencing of the cDNA)

trans-splice product of probe / exploration RNA and cellular pre-mRNA B
(PCR sequencing of the cDNA)

FIG. 15B

step 2: idendification of potential natural cellular trans-splice products



potential, natural generated trans-spliced (and also pathogenous) mRNA
(PCR sequencing of the cDNA)

principle of identification of yet unknown cellular mRNA trans-splice products

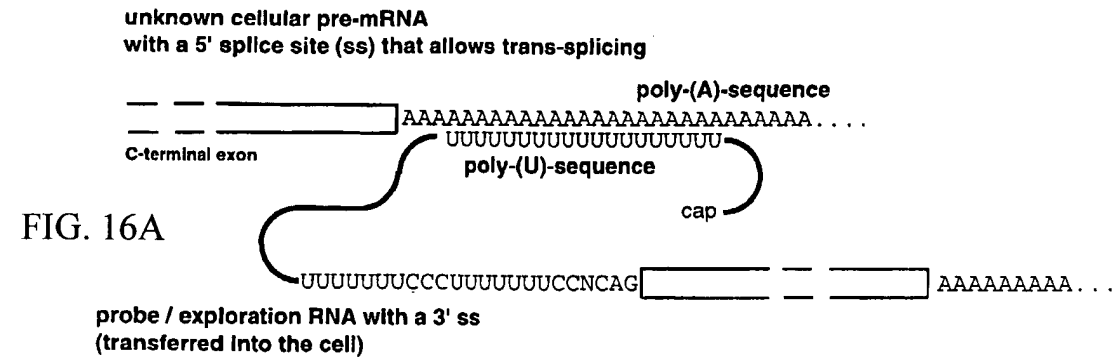


FIG. 16A

association between a probe / exploration RNA and a cellular pre-mRNA that allows trans-spling by antisense pairing between a poly-U-region on the probe / exploration RNA and the poly-A-tail-region on the cellular pre-mRNA

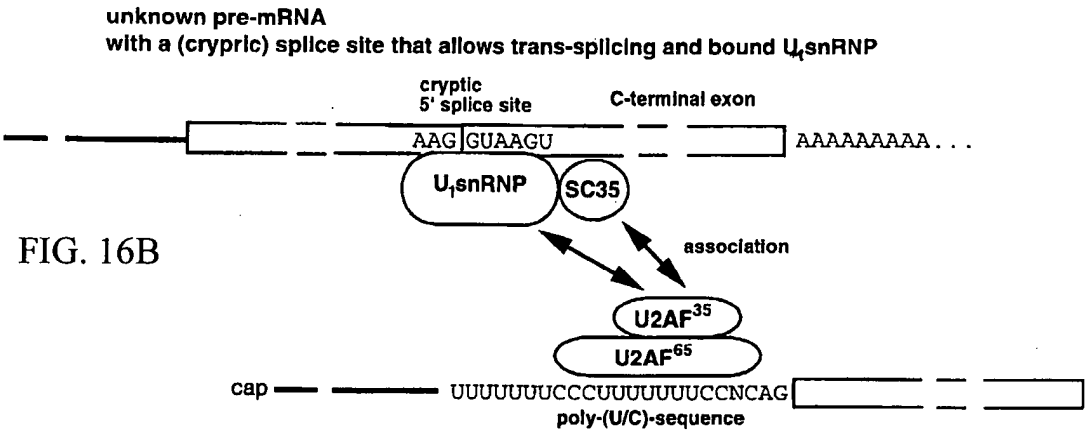


FIG. 16B

association of the 3' splice site on a probe / exploration RNA
to a cryptic 5' splice site in a cellular pre-mRNA that allows trans-splicing
by previously bound splice proteins in the E-complex

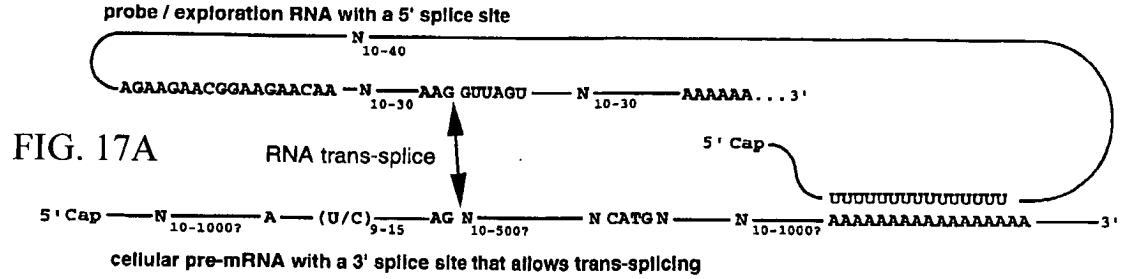


FIG. 17A

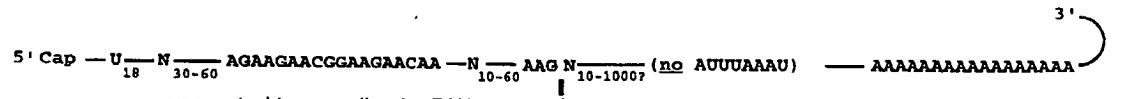


FIG. 17B generated trans-spliced mRNA

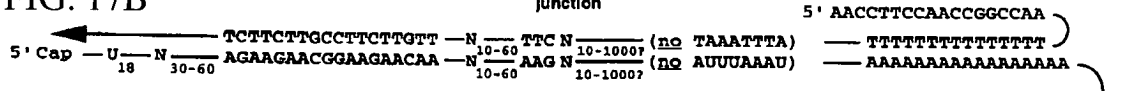


FIG. 17C cDNA-synthesis with primer: 5' AACCGGCCAACCGGCCAA —TTTTTTTTTTTTTTT 3'

FIG. 17D 2nd strand ds-cDNA synthesis (1 x PCR) with primer: 5' AGAAGAACGGAAGAACAA

digest with Swa I: no cleavage possible (no ATTT/AAAT)

FIG. 17E

FIG. 17F so far uncleaved ds-cDNA

FIG. 17G PCR with primer 5' AGAAGAACGGAAGAACAA and with primer 5' AACCTTCCAACCGGCCAA

FIG. (case of unspliced 17H probe / explorat. RNA) sequencing with primer AGAAGAACGGAAGAACAA

FIG. 17I

DNA cleavage with Swa I

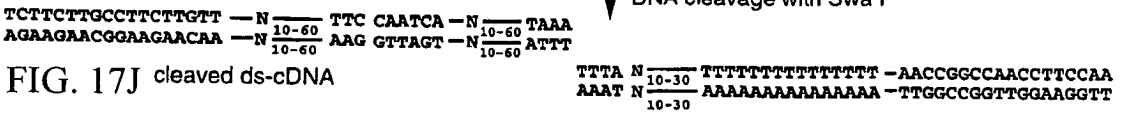


FIG. 17J cleaved ds-cDNA

PCR with primer AGAAGAACGGAAGAACAA and primer AACCTTCCAACCGGCCAA is unpossible after DNA cleavage

FIG. 17K

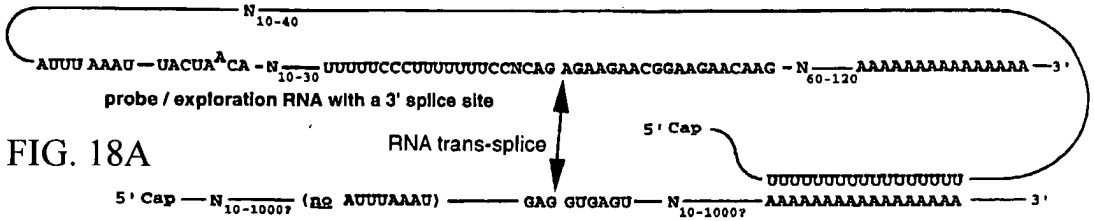


FIG. 18A

FIG. 18B generated trans-spliced mRNA

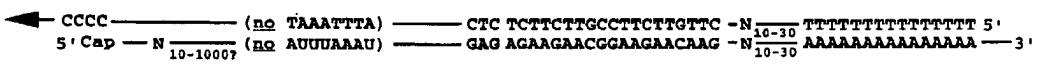


FIG. 18C cDNA-synthesis with primer: TTTTTTTTTTTTTT and after that terminal elongation with cytosins (C)

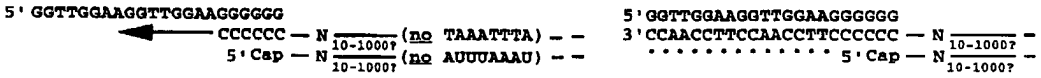


FIG. 18D annealing of primer: GGTTGGAAGGTTGGAAGGGGGG

FIG. 18E complete nucleot. completion to the primer sequence

FIG. 18F 2nd strand ds-cDNA synthes. (1 x PCR) with primer: 5' GGTTGGAAGGTTGGAAG

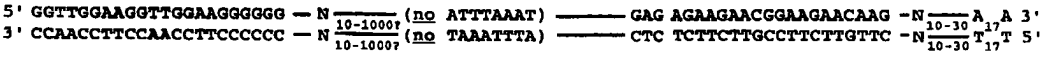


FIG. 18G digest with Swa I: no cleavage possible (no ATTT/AAAT)

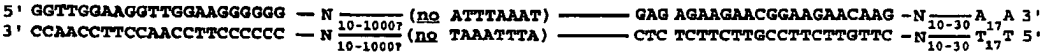


FIG. 18H so far uncleaved ds-cDNA

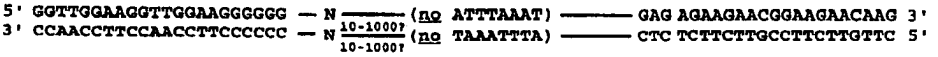


FIG. 18I PCR with primer 5' GGTTGGAAGGTTGGAAG and with primer 5' CTTGTTCTTCCGTTCTTCT

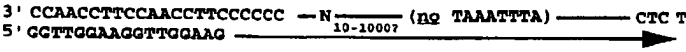


FIG. 18J sequencing with primer GGTTGGAAGGTTGGAAG

(case of unspliced probe / explorat. RNA)

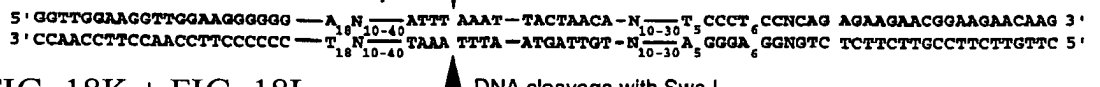


FIG. 18K + FIG. 18L DNA cleavage with Swa I

PCR with primer GGTTGGAAGGTTGGAAG and primer CTTGTTCTTCCGTTCTTCT is unpossible after DNA cleavage

FIG. 18M

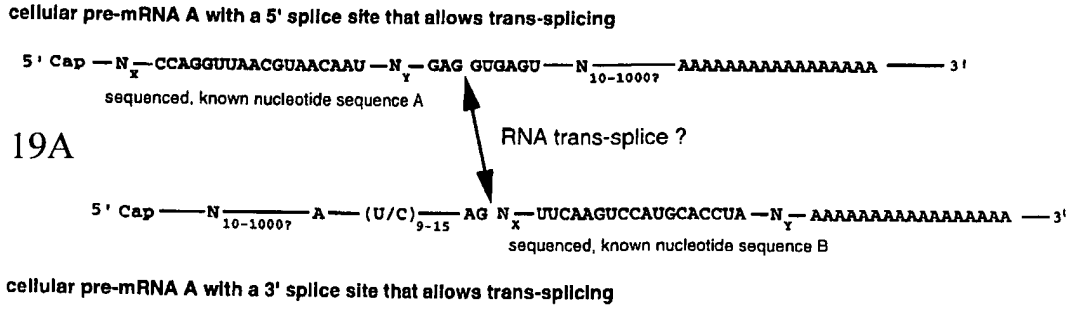


FIG. 19A

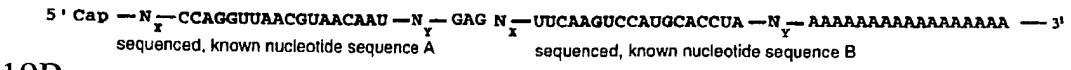


FIG. 19B if RNA trans-splice occurs in vivo: generated cellular hybrid mRNA

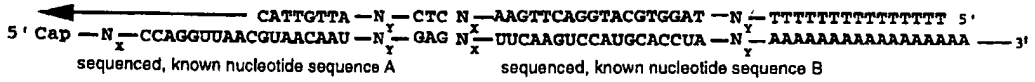


FIG. 19C cDNA synthesis with primer TTTTTTTTTTTTTTT

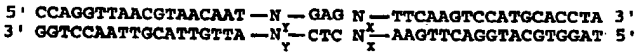


FIG. 19D PCR with primer 5' CCAGGTTAACGTAACAAT and primer 5' TAGGTGCATGGACTTGAA

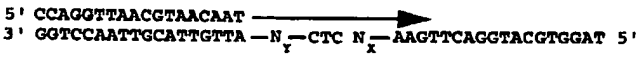


FIG. 19E confirming sequencing with primer CCAGGTTAACGTAACAAT

final evidence on natural cellular trans-splice products
generated by trans-splicing between two pre-mRNAs that both allow trans-splicing